

THE UNIVERSITY OF CHICAGO.

FOUNDED BY JOHN D. ROCKEFELLER.

THE EFFECTS OF IONS UPON THE AGGREGATION
OF FLAGELLATED INFUSORIA.

BY

WALTER E. GARREY.

A DISSERTATION

SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS,
LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (DEPARTMENT OF PHYSIOLOGY).

1900.

THE EFFECTS OF IONS UPON THE AGGREGATION OF FLAGELLATED INFUSORIA.

BY WALTER E. GARREY.

[From the Hull Physiological Laboratory of the University of Chicago.]

CONTENTS.

	Page
I. The Aggregation of Infusoria, How Produced	291
Introduction	291
The organism (<i>Chilomonas</i>)	293
The method	294
The effects of hydrochloric acid	295
The effects of weak lactic acid	297
II. The Effects of Ions upon the Aggregation of Flagellates	298
The use of chemically equivalent solutions	298
The effects of alkalies	299
The inorganic acids	300
The salts of inorganic acids	302
The organic acids	306
Positive chemotropism to organic acids	310
The effects of salts of organic acids	312
Summary	314

I. THE AGGREGATION OF INFUSORIA, HOW PRODUCED.

Introduction.—This investigation was undertaken at Professor Loeb's request and under his direction, with the object of determining to what extent the gathering of infusoria subjected to the influence of solutions of electrolytes was determined by ions, and with the hope of establishing an analogy between the effects of chemicals and the effects of other stimuli upon the motions of these organisms.

Many writers have failed to recognize the fact that gatherings of motile forms subjected to a given stimulus are not always due to the same effect of the stimulus. Dr. Loeb has shown that light may cause gatherings of animals in two ways:—

I. By a reaction of the animal to *sudden changes in the intensity of illumination*, effecting gatherings in the less intensely illuminated area. Such animals Loeb calls "*Unterschiedsempfindlich*,"¹ photokinetic.²

¹ LOEB, J.: Archiv f. d. ges. Physiol., 1893, liv, p. 81.

² The term "photokinesis" (photokinetic) does not express adequately the meaning of the term "*Unterschiedsempfindlichkeit*," but expresses the essential feature of the

II. By an orientation of the animal such that its axis of symmetry coincides with the direction of the rays of light, with a consequent motion to or from the source of light. Such animals Loeb calls "heliotropic."

If a vessel containing various forms of animals representing the above mentioned forms of sensitiveness to light be placed before a window (Fig. 1., *A-B*), it soon becomes

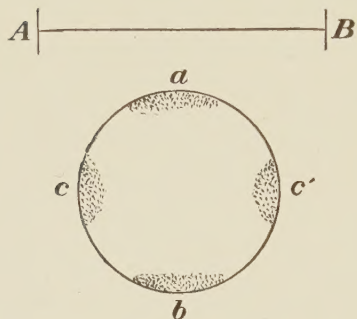


FIGURE 1.

evident that there is a gathering of certain forms on the side nearest the window (Fig. 1, *a*). Such forms are said to be *positively* heliotropic (*e. g.* plant lice). Other forms, which are *negatively* heliotropic (*e. g.* larvæ of the fly), gather on the side farthest from the window (Fig. 1, *b*).

Still other forms gather along the sides of the dish where the intensity of illumination is least, *e. g.* fresh-water planarians (Fig. 1, *c* and *c'*). These forms are photokinetic (*Unterschiedsempfindlich*). They become restless if the intensity of the light is suddenly increased, while they become quiet ("fall asleep") if the intensity is suddenly decreased. If they creep about and come into an area where the intensity of the light is comparatively small, they become quiet. Hence those places where the light is a comparative minimum act like traps in which the planarians are caught, and consequently gatherings take place in these less intensely illuminated areas. Besides planarians, earthworms and many other animals show this reaction.

Loeb has pointed out that *every true tropism is due to an orientation* of the organisms. In heliotropism the organism is so oriented that its axis or plane of symmetry coincides with the direction of the rays and symmetrical points on the surface of the body are struck by the light rays at the same angle. The direction of the movement is determined by the direction of the rays of light. In galvanotropism the current curves, that is, the path of migration of the ions, determines the direction of movement.

phenomenon to be described. Davenport proposes the term photopathy, which does not express the increase of motion, and in addition is suggestive of suffering. As a substitute for the phrase "*Unterschiedsempfindlichkeit* to chemicals" I have introduced the term "chemokinesis" and call organisms showing this reaction "chemokinetic."

In chemotropism Loeb introduces the conception of lines of diffusion, *e. g.* the lines along which the diffusing molecules or ions move from a centre of diffusion. The lines of diffusion play the same rôle in chemotropism that the rays of light do in heliotropism or the current curves in galvanotropism.

Loeb expresses the possibility of a perfect analogy of chemotropism with the two other forms of tropism, in these words: "The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle. Thus symmetrical muscles are in the same state of tension."¹

If we wish to analyze the reactions of animals to chemicals, we must answer the following questions: Is there a *chemokinesis*? Is there a true chemotropism?

This investigation resulted in an affirmative answer to these questions, and in an establishment of the analogy between the effects of

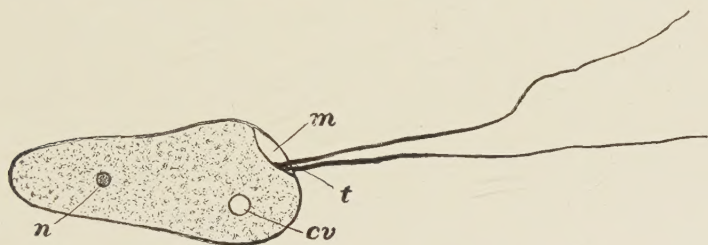


FIGURE 2.

chemicals and the effects of other stimuli which cause gatherings of infusoria. Gatherings take place which are *due to true chemotropism*. The organisms move toward the centre of diffusion of certain chemicals (*e. g.*, weak lactic acid) as if orienting themselves to radially disposed lines; lines which may represent the paths of diffusing molecules or ions. Furthermore, other gatherings of infusoria occur which are *not due to such an orientation*, but to an effect of a change in the concentration of the chemical used (*e. g.*, an inorganic acid) to *chemokinesis*, a reaction completely analogous to photokinesis. A more detailed account of the two kinds of reaction of the infusoria will follow the description of the organism used and of the method employed.

The organism. — *Chilomonas*, the organism chosen for this investi-

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1897, lxvi, p. 442.

gation, shows most marked reaction to chemicals in solution. The cultures were obtained by soaking ordinary peat moss obtained from the Lake Superior region. The single individual is not visible to the naked eye. Under the microscope it presents somewhat the appearance of a diminutive pear, at the broad (anterior) end of which two flagella, twice the length of the body, may be seen (Fig. 2). At the base of these flagella is a funnel-shaped depression, the mouth (*m*). A single contractile vacuole (*cv*) is situated near the mouth. In the posterior half may be found the nucleus (*n*). The whole body presents a coarsely granular appearance.

The method. — The determination of the exact nature of the reactions of the organism was found impossible by the methods heretofore employed, *i. e.*, by introducing into some of the culture capillary

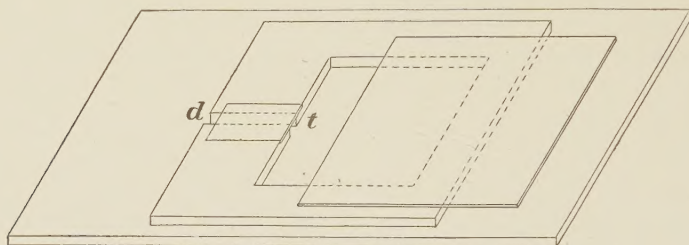


FIGURE 3.

tubes containing the solution to be tested, or by introducing drops of the solution. The device shown in Fig. 3 was used throughout this investigation, and gave most satisfactory results. It consists of a chamber about 1 or $1\frac{1}{2}$ mm. deep, made by fastening hard rubber to a glass slide with sealing-wax. An opening $1\frac{1}{2}$ mm. wide is left in one side of the border about this chamber. This opening is covered by a piece of cover-glass countersunk flush with the surface. The tube thus formed, which will be called the diffusion tube (Fig. 3, *dt*), can be filled by capillarity with the solution to be tested. A cover-glass is pushed over the chamber from the side opposite this tube, and the chamber is filled with the culture as rapidly as it is covered. The culture and the solution in the tube come in contact at the instant the chamber is full and covered.

At the instant of contact between the solution in the tube and the culture in the chamber, the solution will begin to diffuse. The behavior of the organism under the influence of the solution may then

be studied.¹ Let us consider the phenomena in some such cases, noting some details of the reactions which serve to support the assertion already made as to the analogies existing between reactions of infusoria toward chemicals and toward other forms of stimulation. From this study we may also obtain the criteria for the determination of the effects of various ions.

Effects of hydrochloric acid.—If a solution of hydrochloric acid be the solution in the diffusion tube, and the chamber be filled with *Chilomonas* culture, it is noticed that the organisms leave the area

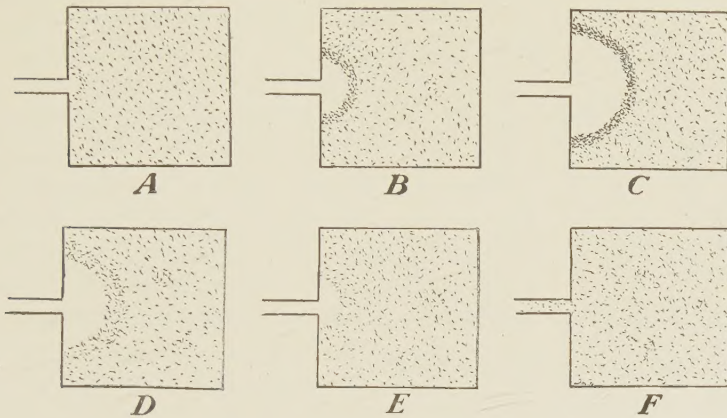


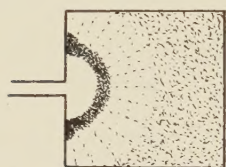
FIGURE 4.

surrounding the mouth of the diffusion tube (Fig. 4, *A*). A semi-circular area in which there are no organisms results. This area gradually becomes larger and is bounded by a ring in which the organisms are more densely gathered than they are in the surrounding culture medium (Fig. 4, *B*). This ring keeps pace with the enlarging clear area (Fig. 4, *C*). The enlargement continues, and if the acid is strong the ring is pushed to the periphery of the dish where the organisms scatter or die. If, however, the acid is not strong, the enlargement of the ring and clear area may stop sooner, in which case the ring slowly disappears (Fig. 4, *D*). The clear area, now un-

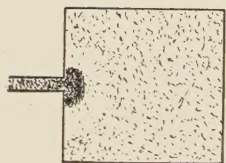
¹ In the experiments with *Chilomonas* much depends upon the conditions in the culture fluid. In order to equalize the conditions some of a dense culture of infusoria was diluted with a large amount of water before being submitted to an experiment. After this washing process constant results could be obtained. No experiment was recorded without experimental evidence that I could *not* obtain the same result with distilled water in the diffusion tube.

bounded by a ring, diminishes in size (Fig. 4, *E*), and finally recedes to the diffusion tube (Fig. 4, *F*), and no traces of the acid's action are apparent.

Are the formation of the ring and the clear area due to chemotropism or to chemokinesis? Observations showed that acids above a certain concentration cause the organism to become restless, very swift shooting movements being induced. The concentration of the acid varies inversely as the square of the distance from the mouth of the diffusion tube. At a certain distance from the mouth of the tube there is a line at which the acid becomes too weak to cause this comparative restlessness. Organisms moving from the area containing the strong acid and crossing this critical line at once



A



B

FIGURE 5.

become relatively quiet. The space just outside the critical line therefore acts like a trap in which the organisms are caught. In the course of time the critical line will move farther and farther from the mouth of the diffusion tube, necessarily causing the entrapped organisms to move with it. It is evident from these considerations that toward strong hydrochloric acid *Chilomonas* is chemokinetic. That, in the zone surrounding the clear area there is a dense gathering (in other words, that there is a ring formation), is in my opinion due to the fact that those individuals which were in the clear area are now gathered in the space immediately surrounding it. It might be argued that in

addition to this, another cause contributes to the formation of the ring, namely, that the weak acids might have an effect the reverse of that produced by strong acids. This would cause the infusoria to leave the periphery of the chamber and to gather nearer the critical line. Jennings indeed maintains that *Paramecia* are negative to strong acids and positive to weak acids, and thus explains the ring formation. But this is not the case for *Chilomonas*. I have never seen *Chilomonas* move toward an inorganic acid. If the acid is too weak to cause a clear area, no gathering ever takes place.

The fact that *Chilomonas* does not move *toward* an inorganic acid (*i. e.*, is neither chemokinetic nor positively chemotropic to *inorganic* acids) explains why the ring disappears and does not follow the clear

area in those experiments where the acid was too weak to cause the ring to move back to the periphery, and in which the clear area was seen to diminish in size after reaching a maximum (see page 295, Fig. 4, *D* and *E*).

The effects of lactic acid. — If lactic acid be substituted for hydrochloric acid the general behavior is somewhat different. A clear area bounded by a ring appears about the mouth of the diffusion tube just as in the case of hydrochloric acid, but the area does not increase in size as rapidly as when hydrochloric acid is employed. It is almost instantly surrounded by a ring in which the organisms are very densely gathered, much more densely than in the case of hydrochloric acid (Fig. 5, *A*). This ring does not disappear when the clear area has reached its maximum size, as in the case of hydrochloric acid; but when the clear area begins to recede again, the ring keeps pace with the change; and finally when the clear area has disappeared, the organisms of the ring form a dense plug in and about the mouth of the tube (Fig. 5, *B*).

How are these phenomena to be explained? I believe they are due to the fact that while for strong lactic acid the organisms are chemokinetic, for weaker lactic acid they are positively chemotropic.

A chemokinesis to strong lactic acid explains why a clear area is formed at the mouth of the tube and why the clear area increases in size. The second assumption, of a positive chemotropism to weaker lactic acid, explains why, after the acid has diffused until its concentration has fallen below a certain degree, the clear area and the ring become smaller and recede toward the mouth of the tube until they reach it.

That the organisms are positively chemotropic is decided by the following observation: —

Immediately outside the dense ring or gathering there is an area which shows a diminished number of organisms. It is easy to notice that the organisms within this area are oriented with the anterior ends (those bearing flagella) directed toward the ring, and that they are swimming in strictly radial lines, the lines of diffusion, toward the diffusing drop.

Hence those phenomena which indicate a *positive chemotropism* are produced by weak lactic acid.

II. THE EFFECTS OF IONS UPON THE AGGREGATION OF FLAGELLATES.

The use of chemically equivalent solutions.—In determining the effects of ions upon the movements of flagellates, Arrhenius's theory of electrolytic dissociation was necessarily used as a working basis. The older investigations of chemotropism, *e. g.*, those of Pfeffer,¹ did not deal with our problem for the simple reason that the dissociation of electrolytes was not yet known.

In making determinations of the chemotropic effects of ions equal percentage solutions cannot be compared. Yet even in recent literature such comparisons occur. For example, Jennings² compares the chemotropic effects of equal percentage solutions of various salts (LiCl, KCl, and others), and from the comparison draws the erroneous conclusion that lithium salts are "more repellent" than potassium salts, and that "the repellent power varies inversely with the atomic weight of the metal components." Jennings, to be sure, recognized the fact that a larger number of molecules of the lighter salt went into the solution, but did not see that this very fact contradicted the conclusions he had drawn. In addition, he did not consider the fact that in the solutions with which he worked the molecules were dissociated into ions; yet the ion concentration must be considered. If dissociation is complete, the effects produced by the solutions are the effects of all or of one class of the ions in the solutions. If the dissociation is not complete, the degree of dissociation must be determined.³

¹ PFEFFER: Untersuchungen aus dem botanischen Institut zu Tübingen, 1881-1885, i, p. 363.

² JENNINGS, H. S.: This journal, 1899, ii, p. 374.

³ To designate chemically equivalent solutions, the chemist uses fractional parts of the so-called "normal" solutions, *i. e.*, the solution made by dissolving the equivalent gram-molecule in one litre of water; *e. g.*, 36.5 grams of HCl per litre, or 3.65 per cent, is the normal solution; of H₂SO₄ (a dibasic acid), $\frac{98}{2}$ grams per litre, or 4.9 per cent is the normal solution. If to 1 c.c. of a normal solution 99 c.c. of water be added, the dilution (V) = 100 and the fraction $\frac{N}{100}$ represents the strength of the dilute solution. If the dissociation is not complete, the degree of dissociation (a) can be computed from the electrical conductivity, $a = \frac{\mu_v}{\mu_\infty}$, in which equation μ_v represents the electrical conductivity at the dilution V (*i. e.* $\frac{N}{V}$) and μ_∞ the conductivity when $V = \infty$, *i. e.*, when the dilution is infinite. The sum of the velocities of the ions for a given temperature gives μ_∞ .

The Alkalies.¹ — The specific chemical action of alkalies is due to the hydroxyl-ions. It is our intention to find out whether it is also the OH-*ion* which causes the reactions of infusoria subjected to the influence of alkalies.

The following alkalies were tested: LiOH, NaOH, KOH, Ca (OH)₂, Sr (OH)₂, Ba (OH)₂.

If these hydroxides act upon cultures of *Chilomonas* they invariably cause a clear area about the mouth of the tube when the solutions are as strong as $\frac{N}{500}$, and usually when they are considerably more dilute. At these dilutions the alkalies are completely dissociated into hydroxyl (OH-) ions (anions), and metal- or basic-ions (kations). The effect produced is therefore due solely to the action of ions; whether to the action of OH-ions or kations is decided by the following fact: $\frac{N}{500}$ solutions of the corresponding chlorides, and even solutions of three times this strength, produce no apparent effect upon *Chilomonas*. Such solutions of the chlorides are completely dissociated. It follows then that the formation of the clear area about the mouth of the tube by the action of alkalies as dilute as $\frac{N}{500}$ is due to the action of the *hydroxyl-ions*. This deduction is further supported by the fact that no difference can be distinguished in the phenomena presented by *Chilomonas* cultures subjected to the actions of chemically equivalent solutions of the different alkalies. Barium hydroxide seems to act in precisely the same manner and with the same intensity as sodium hydroxide, provided the concentration of OH-ions is the same in both cases.

It has already been stated that $\frac{N}{500}$ and stronger solutions of the alkalies invariably cause the clear area about the mouth of the tube. $\frac{N}{1000}$ solutions are strong enough to do this in most cases, while at times the organism is sensitive to solutions as dilute as $\frac{N}{2000}$. This variation is due to the different sensitiveness of the infusoria of different cultures and to the fact that the sensitiveness of the organism in any culture varies from day to day. If the same culture be employed in all the experiments of a single day, the results are constant.

If a strong solution of alkali is tested, a ring is formed similar to that formed about diffusing hydrochloric acid and already described. In this connection no orientation of the infusoria can be detected. The ring is only temporary and cannot be produced by dilute solutions. From these facts I conclude that *Chilomonas* is chemokinetic to OH-ions.

¹ Since strong alkalies attack hard rubber the experiments were repeated in an apparatus made entirely of glass.

Solutions of the carbonates of those bases whose hydroxides were tested contain free hydroxyl-ions. These carbonates in our experiments acted like the hydroxides. The conditions in such solutions are too complex to allow the establishment of any quantitative relations; I can only state that the typical clear area appeared with solutions of Na_2CO_3 , K_2CO_3 , and other carbonates so dilute that chemically equivalent solutions of the chlorides of corresponding metals produced no effect. The effects produced by the carbonates then are not due to the kations, but are due either to the hydroxyl-ions or to CO_3 -ions or to the undissociated molecules.

The behavior of *Chilomonas* toward NH_4OH was also tested. The experiments yielded precisely the same quantitative results as did those with the other alkalis. Due to incomplete dissociation, however, the number of OH-ions is much smaller in solutions of NH_4OH than in chemically equivalent solutions of the other alkalis. The effect, then, cannot be due to the OH-ions alone, but there is probably added to it the effect of the NH_4 -ions or the undissociated NH_4OH molecules, or both.

Inorganic acids.—The common properties of acid solutions are due to the hydrogen-ions present. This statement is true for general chemical reactions, as well as for the effects obtained when *Chilomonas* cultures are subjected to the action of chemically equivalent solutions of HCl , HNO_3 , or H_2SO_4 . The phenomena observable have already been described in the general discussion of the reactions of the organism to HCl . It remains to consider the action of *ions* in producing the phenomena.

The characteristic clear area appears about the mouth of the diffusion tube whenever solutions of these inorganic acids as strong as $\frac{N}{10000}$ are tested. At the dilution $\frac{N}{10000}$ each of these acids is completely dissociated into H-ions and the anions characteristic of the acid.

Is the effect due to the H-ions or to the anions? To decide this, experiments were made with salts that contained the same anion as the acid. It was found that a chemically equivalent solution of the sodium salt of any of these acids, or even a solution ten times as strong, had no effect upon *Chilomonas*. Since in these solutions the salt is completely dissociated and the anions produce no effect, the effects produced by the acids can be due only to the H-ions.

As was noted in considering the effects of the hydroxyl-ion, *Chilomonas* does not always exhibit the same sensitiveness in its reaction

toward the same ions. The hydrogen-ion invariably causes the clear space when the solution of the inorganic acid is $\frac{N}{1000}$, and sometimes when as dilute as $\frac{N}{3000}$. If the solution in the tube was $\frac{N}{4000}$ or $\frac{N}{5000}$ the organism showed a sensitiveness only at the instant of contact with it. The most common dilution of the inorganic acids beyond which the clear area was not formed, was about $\frac{N}{1500}$ to $\frac{N}{1800}$.

If a comparison be made between the action of the H-ion and OH-ion, it is evident that the H-ion is the more active. The ratio of activity is about 2:1. If a solution of mineral acid be so diluted that the clear area is barely evident, it will require a solution of alkali almost twice as strong to produce the same effect. This fact is significant when considered in the light of Loeb's observation that within the various groups of the natural system the poisonous effects of ions upon muscle are, to a certain extent, a function of their velocity.¹ The ratio of the velocity of the H-ion to that of the OH-ion is, according to Ostwald, 325 to 170, when the temperature is 25° C. This ratio is nearly 2:1, or the same as their effect upon Chilomonas. Jennings states that in his experiments with Paramecia "the results with acids (inorganic) are not due to the hydrogen-ion of the acids," but to the anions.² The acids used by Jennings were completely dissociated, and the hydrogen-ions are the only ones common to all the acids. The anions cannot produce the effects as will be further shown in the discussion of the effects of inorganic salts, and we are forced to the conclusion that *it is the H-ion of inorganic acids* which produces the observed effects.

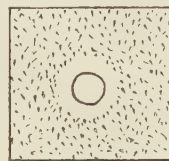


FIGURE 6.

Solutions of carbon dioxide have acid reactions due to the fact that free hydrogen-ions are formed by the electrolytic dissociation of carbonic acid (H_2CO_3). It would be expected, *a priori*, that the behavior of Chilomonas toward a bubble of CO_2 or, more exactly, toward the carbonic acid solution surrounding the bubble, would be similar to that toward *weak* solutions of other inorganic acids. This is, in fact, the case. A clear area forms about the bubble at a short distance from it, as is shown diagrammatically in Fig. 6. The organism does not at any time swim to the bubble as if attracted by it. No

¹ LOEB, J.: Archiv f. d. ges. Physiol., 1897, lxix, p. 21.

² JENNINGS, H. S.: This journal, 1899, ii, p. 370.

ring or gathering of any kind is noticeable.¹ Aqueous solutions of carbon dioxide introduced into the diffusion tube had the same effect upon *Chilomonas* as the bubble of CO_2 or as *weak* solutions of the other inorganic acids. This effect of H_2CO_3 in a solution of CO_2 is probably due largely to H^+ -ions, but since the degree of electrolytic dissociation of H_2CO_3 is not high, the molecules of the acid may play a large rôle. Another factor may be CO_2 molecules in simple solution.

Salts of inorganic acids.—The relative action of the ions of the inorganic salts was determined by testing groups of these salts in series, *e. g.*, to find the relative action of the Li^- , Na^- , and K^- -ions, series of chlorides of these bases were tested. Chemically equivalent solutions of these salts have the same number of chlorine ions, and differences in the reactions of *Chilomonas* to these solutions are due to differences in the specific activities of the kations. The relative effects of the kations can be determined by comparing various salts of inorganic acids. Similarly, the effects of the anions may be determined by comparing the Li -salts or Na -salts or K -salts of the various acids. In trying to determine the effects of anions it is expedient to select salts in which the kation is comparatively ineffective, and *vice versa*.

To determine the action of the anions Cl , Br , and I , series of the lithium salts, sodium salts, and potassium salts were separately tested. In every case no effect was produced upon *Chilomonas* cultures except the formation of the clear area about the mouth of the diffusion tube in which the salts were placed. If $\frac{N}{10}$ NaCl was gradually diluted until the solution no longer produced the clear area, it was found that

¹ These investigations with *Chilomonas* agree with the investigations of Loeb and Hardesty,¹ who found that *Paramecium* left those regions where CO_2 was entering into solution. Jennings claimed to have found *Paramecium* positively "attracted" to CO_2 .²

I made series of experiments with *Paramecia* (taken directly from the culture, and also washed with distilled water) with the intention of confirming Jennings's statement. I introduced CO_2 bubbles under a cover-slip; in another experiment the gas was led through the Engelmann chamber in which *Paramecia* were observed; while in a third series I tried the effects of water saturated with H_2CO_3 —but always with a result contrary to that of Jennings. The *Paramecia* were not "attracted" to CO_2 . Carbon dioxide acts upon *Paramecium* as it does upon *Chilomonas*, *i. e.*, just as all inorganic acids do.

¹ LOEB, J., and I. HARDESTY: *Archiv f. d. ges. Physiol.*, 1895, lxi, p. 591.

² JENNINGS, H. S.: *Journal of physiology*, 1897, xxi, p. 288 et seq.

chemically equivalent solutions of NaBr and NaI were still strong enough to produce it. At a dilution at which the NaBr ceased to have any perceptible effect upon the organism of the culture, NaI still caused the formation of the clear area. The lithium and potassium series gave results similar to those obtained with the sodium series. The table below gives the results of some typical experiments. The strengths given represent the dilution at which the clear area was no longer formed.

TABLE I.

	Cl.	Br.	I.
Li . . .	$\frac{N}{17}$	$\frac{N}{33}$	$\frac{N}{53}$
Na . . .	$\frac{N}{20}$	$\frac{N}{33}$	$\frac{N}{55}$
K . . .	$\frac{N}{35}$	$\frac{N}{50}$	$\frac{N}{80}$

At the dilutions given in the table, the salts may be considered completely dissociated, and any effect will be due to the action of the ions. In the less dilute solutions convection currents were noticeable and it was thought that the formation of the clear area might have been due to some physical action of the solution dependent upon the specific gravity. The comparison of the strengths at which perceptible effects ceased shows that the action of the solutions is not proportional to the specific gravities. To test the question further the action of solutions of glucose toward the organism was examined. Solutions of glucose with a specific gravity greater than that of any of the solutions of salts mentioned in Table I did not cause the formation of the clear area about the mouth of the diffusion tube. Convection currents were noticeable, but no direct effect upon the organism, which was able to swim, though slowly, into the solutions. The conclusion that the effect of the solutions of salts considered above upon *Chilomonas* is a chemical one is justifiable.

The difference of chemically equivalent solutions of the halogen salts of Na, or of Li, or of K is due to the different effects of the ions Cl, Br, and I. Of these ions, Cl is the least active, Br next, and I the most active. The ratio of activity seems to be about Cl:Br:I::2:3:5. The ions are monatomic, and their effect upon *Chilomonas* *increases* with their atomic weight, though it is not necessarily a

function of their atomic weight. Kahlenberg¹ found that the salty taste of the halogen ions *decreases* as the atomic weight increases.

The effect of chemically equivalent solutions of LiCl and NaCl upon *Chilomonas* cultures is about equal. Solutions of KCl will cause the characteristic clear area about the mouth of the diffusion tube at dilutions at which solutions of NaCl or LiCl have no effect. When $\frac{N}{20}$, LiCl, or NaCl no longer have this effect, $\frac{N}{27}$ to $\frac{N}{30}$ KCl is still just able to produce the clear area, a fact which agrees with Loeb's experience.

Comparison of the bromides gives analogous results. KBr causes the formation of the clear area in solutions as dilute as $\frac{N}{40}$ to $\frac{N}{50}$, while solutions of LiBr and NaBr must be as strong as $\frac{N}{30}$ to produce the same effect. These solutions are completely dissociated.

From these facts we draw the conclusion that, so far as *Chilomonas* cultures are concerned, the Li- and Na-ions have equal effect and that the K-ions are more active than the other two.

In order to give the reader an idea of how much the individual experiments vary I add Table II, which gives the result obtained in a series of six experiments with the solutions just considered. This result is typical of a large series. The strengths of the solutions given in the table denote the dilution necessary to just prevent the formation of a clear area when the solution is placed in the diffusion tube.

TABLE II.

	1	2	3	4	5	6
LiCl	$\frac{N}{15}$	$\frac{N}{50}$	$\frac{N}{17}$	$\frac{N}{30}$		$\frac{N}{40}$
NaCl	$\frac{N}{20}$	$\frac{N}{30}$	$\frac{N}{20}$			
KCl	$\frac{N}{27-30}$	$\frac{N}{70}$	$\frac{N}{33}$	$\frac{N}{60}$	$\frac{N}{35}$	$\frac{N}{80}$
LiBr	$\frac{N}{30}$	$\frac{N}{80}$	$\frac{N}{33}$	$\frac{N}{80}$		
NaBr	$\frac{N}{30}$	$\frac{N}{73}$	$\frac{N}{32}$			
KBr	$\frac{N}{30-40}$	$\frac{N}{110}$	$\frac{N}{50}$		$\frac{N}{50}$	
LiI				$\frac{N}{50}$		
KI					$\frac{N}{80}$	

¹ KAHLENBERG, L.: Bulletin of the University of Wisconsin, Madison, Sept., 1898, No. 25.

No difference could be detected in the reaction of *Chilomonas* to equimolecular solutions of calcium chloride and strontium chloride. Magnesium chloride was less active than either, producing the same effect only by stronger solutions. Barium chloride was the most active of the chlorides of the alkali earths. If the solutions of the other members of this series were diluted until they just failed to produce an effect on *Chilomonas*, an equivalent solution of barium chloride was markedly active in the formation of the clear area about the mouth of the diffusion tube. Three series of experiments are given in the subjoined table, showing the dilutions at which solutions of the salts cease to produce the formation of the clear area in a culture of *Chilomonas*.

TABLE III.

	1	2	3
MgCl ₂ . . .	$\frac{N}{75}$	$\frac{N}{100}$	$\frac{N}{60}$
CaCl ₂ . . .	$\frac{N}{108}$	$\frac{N}{135}$	$\frac{N}{95}$
SrCl ₂ . . .	$\frac{N}{116}$	$\frac{N}{140}$	$\frac{N}{100}$
BaCl ₂ . . .	$\frac{N}{175}$	$\frac{N}{215}$	$\frac{N}{150}$

A clear area appears in the *Chilomonas* culture about the mouth of the diffusion tube whenever the solutions of the salts are a trifle stronger than the figures in the table indicate. At such strengths the salts may be considered completely dissociated and the effects are those of ions. Differences in the action of these salts can only be due to the different metal constituents. We must not, however, overlook the fact that another kation may co-operate with it. These salts are salts of bivalent metals and the molecule may dissociate in two ways: *e. g.* $MgCl_2 = \overset{+}{Mg}, \bar{Cl}, \bar{Cl}$, or $= \overset{+}{MgCl}, \bar{Cl}$. I cannot state what share each of the two possible kations has in the action of each of the solutions.

The ratio of the effects of chlorides of Mg : Ca : Sr : Ba is 3 : 5 : 5 : 7, nearly.

Comparison between the reaction of *Chilomonas* to solutions of KCl and $\frac{1}{2}$ CaCl₂ showed that calcium chloride in $\frac{N}{80}$ solutions was as effective as $\frac{N}{50}$ solutions of potassium chloride. Ignoring the possibility of the presence and action of the compound kation CaCl,

we may make the statement that the ion Ca has a more marked influence on the reactions of Chilomonas than two ions of K.

The reaction of Chilomonas to solutions of the following salts of the heavy metals was observed: FeCl_2 , ZnSO_4 , ZnCl_2 , CuSO_4 , CuCl_2 , CuCl , HgCl_2 , and AgNO_3 . No effort was made to determine *accurately* the dilution at which reactions to these salts ceased. It was found, however, that these salts in very dilute solutions produced the typical clear area, and often the ring. In most cases the effect followed when the solutions were more dilute than $\frac{1}{10000}$ to $\frac{1}{20000}$. It has already been shown that anions of these salts cannot produce any effect upon Chilomonas when the solutions are as dilute as this. Since the salts are largely dissociated, we conclude that the heavy-metal kations are the active factors which caused the reaction of Chilomonas.

The conditions in some solutions of the heavy metals (*e. g.* CuSO_4) are slightly complicated by the fact that these solutions contain a small number of free H-ions. The effects produced by these solutions simulate those produced by the inorganic acids. Are the free H-ions responsible for the effects? I think not. Suppose that the solution contained as many H-ions as 10 per cent of the number of salt molecules (the number is actually much less than 10 per cent); the acidity of a $\frac{1}{10000}$ solution of one of these salts would then be equivalent to that of a $\frac{1}{100000}$ solution of acid. The characteristic reaction can be obtained with all the above salts in $\frac{1}{10000}$ solutions, but no $\frac{1}{100000}$ acid could produce these effects, and we are forced to conclude that *the effects of such solutions are not, or at least not exclusively, the effects of the free H-ions.*

Jennings concluded from his work on Paramecia that "salts that contain the relatively inactive kation of one of the heavy metals as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence the effect is like that of an acid."¹ My experiments upon Chilomonas show that it is the H-ion, not the anions, that produce the effects of inorganic acids; that the *anions* are relatively inactive; and that *the heavy-metal kations are extremely active.*

Organic acids. — In dealing with the reaction of Chilomonas toward inorganic acids, we showed that the conditions existing in the solutions were comparatively simple. Solutions not strong enough to kill the organism outright were completely dissociated and their effects were due to ions, — in fact, when dilute, could be ascribed to H-ions only. With solutions of organic acids, the conditions are

¹ JENNINGS, H. S.: This journal, 1899, ii, p. 370.

more complex. Dissociation is not complete. Besides the H-ions and complex anions, undissociated molecules are present and undoubtedly play no inconsiderable rôle in the various phenomena seen when the solutions act upon cultures of *Chilomonas*.

Solutions of the following organic acids were experimented with:

I.	II.	III.
Oxalic.	Malic.	Acetic.
Formic.	Tartaric (Dextro).	Lactic.
Citric.	Mandelic.	Butyric.
Succinic.		
Valerianic.		

According to their effect upon the reactions of *Chilomonas*, these acids may be divided for convenience into three groups:—

Group I. The first five acids of the above list comprise the first group. Like the inorganic acids they cause the clear area and, when strong, a temporary and never dense ring about the area. The ring was not formed by a migration of the organism from without to it. No other effects were observable. *Chilomonas* is chemokinetic to these acids.

Group II. Malic, tartaric, and mandelic acids produce a clear area, often with a ring about it. In the formation of the ring, the phenomena were so inconstant that I was unable to say that it was or was not due to a migration of the organisms from without to it. To these acids *Chilomonas* is chemokinetic; whether in addition it is positively chemotropic I cannot say.

Group III. Acetic, lactic, and butyric acids acting upon *Chilomonas* present the phenomena already described in the first part of this paper: a clear area surrounded by a dense ring (see Fig. 5). *Chilomonas* is chemokinetic towards strong solutions of these acids, and is positively chemotropic toward weaker solutions.

The acids of all these groups when sufficiently strong produced the characteristic clear area about the mouth of the diffusion tube noted in all experiments upon *Chilomonas* which have thus far been considered. Inorganic acids as dilute as $\frac{N}{1000}$ or $\frac{N}{1200}$ invariably caused this characteristic clear area, and we concluded that the effects were due to the action of H-ions only. Is the similar effect of organic acids due to the H-ions? If so, we would expect the organic acids to produce the clear area when their solutions were of such strengths that they contained the same number of H-ions as $\frac{N}{1000}$, or $\frac{N}{1200}$ solutions of the inorganic acids.

Oxalic acid never produces the clear area when more dilute than $\frac{N}{700}$. In many cases only solutions stronger than $\frac{N}{300}$ have this effect upon *Chilomonas*. At the strength $\frac{N}{300}$ oxalic acid is largely dissociated and therefore contains about three times as many H-ions as $\frac{N}{1000}$ solutions of inorganic acids like HCl which produce the same effect. The action of the oxalic acid is thus only one third as strong as it should be according to the number of H-ions.

Formic acid is also only about one third as active as is to be expected from its content of H-ions. It requires a solution as strong as $\frac{N}{100}$ to invariably produce the clear area. In $\frac{N}{100}$ formic acid about 33 per cent of the molecules are dissociated. It contains about the same number of H-ions as a $\frac{N}{300}$ solution of an inorganic acid, *i. e.*, more than three times as many as a $\frac{N}{1000}$ solution of an inorganic acid which produces an equivalent effect. In $\frac{N}{300}$ tartaric (dextro) acid about 25 per cent of the molecules are dissociated, and so far as H-ions are concerned, it equals $\frac{N}{1200}$ solutions of inorganic acids like HCl, and produces about the same effects as they do.

On account of the dense ring about the clear area it is not easy to determine with certainty that degree of dilution of acetic acid which no longer causes the formation of the clear area. The following figures are selected to show the variations on different days:

TABLE IV.

1	2	3	4	5	6	7	8
$\frac{N}{300}$	$\frac{N}{300}$	$\frac{N}{200-400}$	$\frac{N}{300-400}$	$\frac{N}{400}$	$\frac{N}{300}$	$\frac{N}{200-400}$	$\frac{N}{300-500}$

It is seen from these data that the clear area is usually formed as a result of the action of acetic acid on *Chilomonas* cultures when the solution is as dilute as $\frac{N}{300}$, and it is invariably formed when the solution is as strong as $\frac{N}{200}$. In $\frac{N}{200}$ solutions of acetic acid about 6.2 per cent (18° C.) of the molecules are dissociated, whereas in equimolecular hydrochloric acid about 95 per cent are dissociated. The HCl solutions therefore contain 15.3 times as many H-ions as the acetic acid. If the HCl be diluted to $\frac{N}{1000}$, it will produce the same effect as $\frac{N}{200}$ acetic acid. But even such a solution will contain more than three times as many hydrogen-ions as $\frac{N}{200}$ acetic acid. The action of acetic acid in the formation of the clear area in cultures of *Chilomonas* is therefore more than three times as great as it should be if the H-ions were the *only* factor in the reaction.

In the formation of the clear area, lactic acid is about as active as HCl, the clear area being always formed by $\frac{N}{1000}$ solutions. At this strength and at a temperature of 19° , only 29 per cent of the lactic acid molecules are dissociated, and this acid therefore contains less than one third as many H-ions as the HCl with which it is equally active.

We therefore see that there is a great difference in the effects of organic and inorganic acids upon the reactions of *Chilomonas*. While inorganic acids show the same effects if they contain the same number of H-ions in the unit volume of the solution, the *organic acids do not show a definite relation between the number of H-ions and the effects produced*. This fact corresponds with what Loeb found previously concerning the effects of acids upon muscle. He showed that the addition of small amounts of acid to physiological salt solutions causes the muscle to take up a considerable amount of water. In the case of the inorganic acids (HCl, HNO_3 , H_2SO_4 , KHSO_4 , NaHSO_4) the amount of water taken up in a given time by the same mass of muscle is the same, if the solutions contain the same number of H-ions in the unit volume. But in the effects of organic acids such simple conditions do not exist. Acetic, and still more so, lactic acid acts much more strongly than is to be expected from the degree of dissociation (*i. e.* from the number of free H-ions in the solution).

Loeb¹ suggests that these differences in the behavior of organic and inorganic acids may be due to the fact that those organic acids which are very incompletely dissociated (*e. g.*, acetic and lactic acids) are transformed inside the muscle into products which undergo a greater degree of dissociation.

Richards² and Kahlenberg³ in their investigations on taste, found similar variations in the effects of organic acids. Both attribute the sour taste of acid to the H-ions in the solution. Both, however, found that by comparison with inorganic acids the sour taste of acetic acid was about three times as intense as the number of H-ions warranted. No explanation of these phenomena is offered by them.

Later, in a review of Professor Loeb's work on effects of ions, Ostwald⁴ makes the following suggestion concerning the variations ob-

¹ LOEB, J.: *Archiv f. d. ges. Physiol.*, 1898, lxxi, p. 460.

² RICHARDS, TH.: *American chemical journal*, 1898, xx, p. 121.

³ KAHLBERG, L.: *Bulletin of the University of Wisconsin*, 1898, No. 25.

⁴ OSTWALD: *Zeitschrift für physikalische Chemie*, 1899, xxviii, p. 174.

served when organic acids are used: "The phenomena observed here and with taste sensations would be explained if it were assumed that the cells contain the neutral salts of an acid of medium strength, the anion of which reacts with the hydrogen-ion." Similar transformations may explain the behavior of the organisms in our experiments on the reactions of *Chilomonas* to chemicals.

It has already been pointed out that *Chilomonas* cultures vary in their sensitiveness from day to day, and it was noticed that on those days when the organism was most sensitive toward H-ions, *i. e.*, when the clear area was formed by the action of very dilute inorganic acids, it showed a proportional sensitiveness toward organic acids. The sensitiveness toward ions other than H, *e. g.* Na, or Cl, did not necessarily vary on those days.

Positive chemotropism to organic acids.—We have already mentioned the fact that lactic acid, besides producing the clear area, has the property of causing a positively chemotropic *orientation* of *Chilomonas*. This orientation is also a characteristic effect of acetic and butyric acids and is responsible for dense gatherings of *Chilomonas* subjected to their influence. If the solution of acid is strong, the gathering has the form of a very dense ring surrounding a clear area. If the solution is sufficiently dilute, the clear area is not formed but a dense gathering into the acid in and about the mouth of the diffusion tube results. The organism is oriented just as when put under the influence of a galvanic current,¹ and migrates along radially disposed lines toward the centre of the diffusing drop. This migration was most markedly evident in two or three experiments in which the organisms were gathered very densely about débris situated some distance from the mouth of the diffusion tube. As soon as they came under the influence of the diffusing acid the organisms left the débris and fairly swarmed into the acid. *The gathering toward these organic acids is due to a positive chemotropism*, to an orientation in the lines of diffusion, and a definite movement into the acids.

On account of the minuteness of *Chilomonas* and the consequent difficulty in observing its flagella, a study of the mechanics by which the organism is oriented, or by which it is prevented from moving from the ring into the stronger acid of the clear area, or the weaker acid surrounding the ring, proved fruitless.

The ring obtained with the inorganic acids is only temporary if the acid is not too strong. In the course of a short time all traces of the

¹ I found that the organism moves towards the anode.

acid's action disappear. On the other hand, the ring obtained with acetic, lactic, or butyric acids is more permanent. In the latter case the gathering has been observed under most favorable conditions to persist as long as three hours, at the end of which time the evidence of its influence disappeared as the diffusion caused the acid to become distributed evenly in the chamber.

Those rings which persist some time were observed to split along their entire length, each ring giving rise to two concentric rings (Fig. 7), the inner of which dissolved, leaving only the outer, which retained very nearly its original position and repeated the splitting process.



FIGURE 7.

Three such consecutive splittings have been observed before the ring disappeared. Exactly similar phenomena of splitting were observed in the rings which formed about bubbles of air and which remained intact long enough (six hours). In very dense collections, *e. g.*, about *débris*, the organisms were also seen to move away from the centre of the dense gathering, leaving a small clear area there.

Solutions of acetic, lactic, and butyric acids as dilute as $\frac{N}{4000}$ to $\frac{N}{5000}$ have been observed to cause dense gatherings of *Chilomonas*; *i. e.*, to produce positive chemotropism. Such gatherings are not, however, usually formed by the action of solutions more dilute than $\frac{N}{3000}$.

The point of interest is: Why do acetic, lactic, and butyric acids produce positive chemotropism if they are sufficiently weak, while the other acids produce no such effect? In solutions of these acids which are even as dilute as $\frac{N}{3000}$, less than one fifth of the molecules are dissociated. There are then three possible factors: H-ions, anions, undissociated molecules.

It has already been shown that the H-ion does not cause any positive chemotropism of *Chilomonas*. We have then to decide between the undissociated molecule and the anion as the active agent, though it is not impossible that both may be factors. It is possible to obtain *other* compounds (salts) which do not contain the acid molecules but do *contain the anions* that are contained in the acid solutions. If the anion of lactic, of butyric, or of acetic acids be the cause of the positive chemotropism reaction of *Chilomonas*, it is to be expected that the salts of these three acids will cause the positive chemotropism in the same way that the acids do.

Salts of organic acids.—Actual experiments showed that sodium oxalate, ammonium oxalate, sodium citrate, and sodium tartrate do not cause any positive chemotropism of *Chilomonas*. They act qualitatively like the salts of the inorganic acid. The salts mentioned are salts of those inorganic acids which I placed in Groups I and II, acids which do not cause any positive chemotropism of *Chilomonas*, or concerning the action of which I am uncertain. *The sodium salts of acetic and butyric acids do, however, cause the positive chemotropic reaction of Chilomonas.* When acted upon by solutions of these salts the organisms swim toward the centre of diffusion along radial lines. The migration soon leads to the formation of a ring outside of which there exists a broad area where there are only a few infusoria, all of which are oriented and swimming

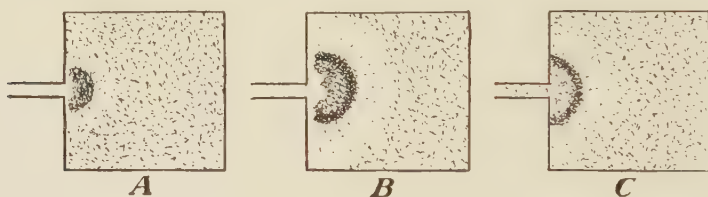


FIGURE 8.

toward the ring. The ring is always densest along its outer border, probably because it is continually receiving additions from the outside and because the ring continually broadens toward the inside. The inner border of the ring is not always sharp and regular as is the case when acids are used. The organisms can move from the densest part of the ring and up into the diffusion tube when the solution within it is as strong as $\frac{N}{8}$. Usually the gathering appears as a patch, which presents a granular aspect due to the uneven distribution of the organisms (Fig. 8, *A*). Very soon this patch is framed by a dense ring (Fig. 8, *B*). If the solutions used are dilute, the gathering has the appearance shown in Fig. 8, *C*.

Chilomonas was positively chemotropic toward solutions of sodium acetate and sodium butyrate which were as dilute as $\frac{N}{40000}$, and in some cases still showed the effects when the solutions were as dilute as $\frac{N}{80000}$. If the anion CH_3COO is the cause of the positive chemotropism of *Chilomonas* to acetic acid or sodium acetate, it is to be expected that the organisms will also be positively chemotropic to other acetates. Solutions of the acetates of copper, iron, and ammonium were tested. *Chilomonas* was positively *chemotropic* toward

all three. In the case of ammonium acetate, acetic acid is present, and the orientation may be due to its action. No quantitative work was done with these salts.

From these facts we conclude that in the case of lactic, acetic, and butyric acids we have two effects upon *Chilomonas*:

1. Chemokinesis produced by the H-ions.

2. *Positive chemotropism* produced by the anions (or by the undissociated molecules).

Pfeffer found that the spermatozoids of ferns were positively chemotropic both to malic acid and its salts. My experiments make it seem highly probable that the effects are due to the anion in the solutions and not to the acid (H-ions) as has thus far been commonly accepted.

Jennings recently expressed the view that "in and of itself there is no positive taxis (tropism) of *Paramecium*" but that all gatherings are brought about by a certain "motor reaction by which it responds to all classes of stimuli" and that the terms "positive and negative taxis (tropism) as applied to *Paramecium* are merely convenient terms for expressing the fact that the animals form collections"¹ or do not. *Orientation and tropism are synonymous expressions*; that *gatherings* take place is a *consequence of the orientation*, and is purely incidental. Jennings's "motor reaction"² cannot account for an orientation, and therefore throws no light whatever upon the tropisms. (Gatherings, however, are not always indicative of a tropism, *i. e.*, of an orientation, but may be due to kinesis (*Unterschiedsempfindlichkeit*), as Loeb showed many years ago, a matter that has already received sufficient notice in the preceding pages of this article).

¹ JENNINGS: This journal, 1899, ii, pp. 311 *et seq.*

² Jennings will pardon me if I call his attention to the fact that the principle of a motor reaction is nothing new. If we apply a sudden local stimulus to a muscle it always reacts with the same motion; it twitches, no matter where we apply the stimulus, and no matter whether the stimulus be electrical, mechanical, or chemical. Likewise, a frog shows the same motor reaction if we suddenly give it an electric shock, or strike it, or touch it with acetic acid; it jumps, as everybody knows, and this same motor reaction occurs, whether the stimulus be applied to the head, to the fore legs, or to the hind legs. The sensitive plant shows a motor reaction; even a human being may show a motor reaction if caught unawares. But all these motor reactions have nothing to do with the *tropisms*, for these motor reactions are only the expression of a very sudden change of the stimuli, while the characteristic of the tropism is the stationary condition of the stimuli.

SUMMARY.

1. There exists a complete analogy between the effects of chemicals upon the reactions of the flagellated infusorian *Chilomonas*, and the effects of other stimuli, *e. g.*, light or the galvanic current, upon certain other forms. We are able to distinguish two causes for the formation of gatherings: first, chemokinesis (*Unterschiedsempfindlichkeit*) and second, positive chemotropism.

2. In determining the effect of ions upon the reactions of organisms, equal percentage solutions must not be compared, but only solutions which have the same ion-concentration.

3. Toward the alkalies LiOH , NaOH , KOH , CaOH_2 , SrOH_2 , BaOH_2 , *Chilomonas* shows chemokinesis and the effect is due to the OH -ions. Chemically equivalent solutions of these alkalies contain the same number of OH -ions per unit volume, and therefore produce equal effects.

4. Toward the inorganic acids, HCl , HNO_3 , H_2SO_4 , *Chilomonas* shows chemokinesis, and the effects are due to the H -ions. If the solutions of these acids have the same concentration of H -ions, they have equal effects. H -ions are more powerful than OH -ions in causing these reactions of *Chilomonas* and apparently in the ratio of the ion velocities.

5. The effect which the carbonates of the so-called alkali-metals and alkali-earths produce upon the reactions of *Chilomonas*, is qualitatively similar to that of the alkalies, and is probably due to the OH -ions, though it may be partly due to CO_3 -ions, or undissociated molecules.

6. Upon the reactions of *Chilomonas* NH_4OH , though very incompletely dissociated, has as marked effects as the other alkalies. To the effects of the OH -ions is added that of some other factors, — NH_4 -ions or NH_4OH -molecules.

7. The effects of carbon dioxide upon *Chilomonas* are really those of carbonic acid (H_2CO_3) and are the same as the effects of other weak inorganic acids.

8. A series of inorganic salts of Fe , Zn , Cu , Hg , and Ag showed that their effect upon the reactions of *Chilomonas* was chemokinetic and due to the kations. Strong solutions of many of these salts contain free H -ions. The effects can, however, be obtained at dilutions which preclude the possibility of an exclusive effect of the H -ions.

9. Toward stimulation by *organic* acids *Chilomonas* shows as a rule chemokinesis. While inorganic acids have equal effects if the ion-concentration is the same, organic acids behave differently. In some cases, *e. g.*, that of acetic acid, the effects are greater than could be expected from the degree of dissociation. This result is similar to that previously found by Loeb on the effects of acids upon the absorption of liquids by muscle, and by Richards and Kahlenberg in their experiments on the taste of acids.

It is possible, as Loeb and Ostwald suggest, that within the tissue the organic acids are transformed into other compounds; the products of these transformations might account for the differences in the effects of organic and inorganic acids.

10. If we use very dilute solutions of certain organic acids, for example dilute solutions of acetic, lactic, and butyric acids, we find that they produce altogether different effects than those produced by solutions of any of the other compounds thus far considered. Toward these solutions *Chilomonas* is positively chemotropic. These positively chemotropic effects are due to the action of the anions or to the undissociated molecules. I was able to show that salts of acetic acid produced positively chemotropic effects when the dilution corresponded to that dilution of acetic acid which produced the positively chemotropic effects. This supports the conclusion that the effect is due to the anion.

I desire to thank Professor Loeb, not only for pointing out the possibilities in this line of investigation and for valuable suggestions as to methods of experimentation, but also for his kindly encouragement, which has made my task a most pleasant one.

